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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Peter BAUMANN, *et al.*
Title:	PROTECTION-OF-TELOMERE-1 (POT-1) PROTEIN AND ENCODING POLYNUCLEOTIDES
Appl. No.:	09/816,248
Filing Date:	March 26, 2001
Examiner:	Unassigned
Art Unit:	1646

AMENDMENT IN RESPONSE TO NOTICE TO COMPLY WITH SEQUENCE RULES

Commissioner for Patents
Box SEQUENCE
Washington, D.C. 20231

Sir:

This is a response to the Notice to Comply mailed June 7, 2001, in the above-identified application. A response is due on August 7, 2001. Although applicants believe that no fees are due, the Commissioner is hereby authorized to credit any overpayment or to charge any deficiency to Deposit Account No. 19-0741. Please amend the application as follows:

IN THE SPECIFICATION:

On page 7, delete the full paragraph beginning with "Figure 4B," and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A marked up version showing changes is attached:

A1 **FIGURE 4B:** Binding of hPot1p to human C-strand (SEQ ID NO: 19) $(CCCTAA)_5$, G-strand (SEQ ID NO: 20) $(TTAGGG)_5$ and duplex (SEQ ID NO: 21)

A1

$(CCCTAA)_5 \bullet (TTAGGG)_5$. Binding conditions and analysis were as described in FIGURE 3.

On page 8, delete the full paragraph beginning with "Figure 6," and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A marked up version showing changes is attached:

A2

FIGURE 6: Inhibition of telomerase activity by Pot1p. Telomerase activity is assayed with telomeric primer PBoli82 (SEQ ID NO: 22) (TGTGGTGTGTGGGTGTGC) as described in Haering *et al.*, *Proc. Nat'l Acad. Sci. USA* 97: 6367-72, 2000. Unlabeled nucleotides are added to a concentration of 100 μM as follows: lanes a and b, dATP, dCTP and dTTP; lanes c and d, ddATP, dCTP and dTTP; lanes e and f, dATP, dCTP and ddTTP. For lanes b, d, and f the oligonucleotide was preincubated with a SpPot1p preparation containing full length protein and the N-terminal 22 kDa fragment (100 ng/μl). The Pot1 protein inhibits primer extension by telomerase.

On page 14, delete the entire Table 1, and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A marked up version showing changes is attached:

TABLE I

SpPot1p-binding oligonucleotides:

(SEQ ID NOS 23-25, respectively, in order of appearance)

A3

Pboli52	GGT TAC GGT TAC AGG TTA CA
Pboli53	CGG TTA CAC GGT TAC AGG T
Pboli54	GTT ACA GGT TAC GGT TAC GG
Pboli86	TGT GGT GTG TGG GTG TGC GGT T
PBoli110	GGT TAC ACG GTT ACA GGT TAC AGG TTA CAG
PBoli112	GGT TAC ACG GTT ACA GGT TAC AGG TTA CAG GGT TAC GGT TAC G
PBoli183	CTG TAA GCA TAT CAT CAT TCG A GGT TAC
PBoli184	GGT TAC GCA TAT CAT CAT TCG A ATC TCG
PBoli185	CTG TAA GCA TAT CAT CGG TTA CGG TTA C
PBoli186	GGT TAC GGT TAC CAT CAT TCG A ATC TCG

PBoli187	CTG TAA GCA TAT GGT TAC TCG A ATC TCG
PBoli188	CTG TAA GC GGT TAC GGT TAC GA ATC TCG
PT1	GGT TAC AGG TTA CAG GTT AC

A3

hPot1p-binding oligonucleotides:

(SEQ ID NOS 36-38, respectively, in order of appearance)

PBoli177	TTA GGG TTA GGG TTA GGG TT
PBoli178	GG TTA GGG TTA GGG TTA GGG
PBoli179	TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG

On page 21, delete the first full paragraph and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A marked up version showing changes is attached:

A4

Specific splicing variants encompassed by the invention are shown in the Figures. The *SpPOT1* gene, for example, has two introns, which normally are spliced from the mature transcript. However, in one splicing variant, intron 2 may not be spliced, so that it is included in the mature transcript (SEQ ID NO:10). Because the intron does not contain a stop codon, the splicing variant mRNA gives rise to a somewhat larger polypeptide (compare SEQ ID NO:9 and 11). When intron 1 is not spliced out, however, the resulting protein is truncated as a result of a stop codon within intron 1. The resulting peptide has the sequence: (SEQ ID NO: 39) M G E D V I D S L Q L N E L L N A G E Y K I G V R Y Q W I Y I C F A N N E K G T Y I S V H. Alternatively, translational frame shifting may lead to a significantly larger protein product. Translational frame shifting has been observed in a number of proteins involved in telomere metabolism. Aigner *et al.*, *EMBO J.* 19: 6230-39, 2000. Polypeptides resulting from translational frame shifting also are considered "splicing variants" for the purposes of the invention.

On page 43, delete the last full paragraph beginning with "*Example 2*:" and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A marked up version showing changes is attached:

Example 2: DNA-binding specificity of SpPot1p.

C-strand (CGTAACCGTAACCCTGTAACCTGTAACCGTGTAACC) (SEQ ID NO: 40) and G-strand (GGTTACACGGTTACAGGTTACAGGTTACAG-GGTTACGGTTACG) (SEQ ID NO: 28) were 5′ ^{32}P-labeled using T4 polynucleotide kinase and γ-^{32}P-ATP. Duplex DNA was generated by annealing equimolar amounts of radiolabeled C-strand and unlabelled G-strand. Binding reactions (10 µl) were carried out in 25 mM HEPES (pH 7.5), 1 mM EDTA, 50 mM NaCl, 5% glycerol, and 2.5 µM PBoli109 (CCGTAAGCATTTCATTATTGGAATTCGAGCTCGTTTTCGA) (SEQ ID NO: 41) as non-specific competitor. Pot1p (50 ng) was incubated with the indicated DNA substrates (1 ng) for 15 min at 20°C. Complexes were analyzed by electrophoresis at 4°C through a 4-20% TBE gel (Invitrogen) run at 150 V for 80 min. The Pot1p-DNA complex is indicated by an open arrow in FIGURE 3A. FIGURE 3B shows the same experiment except that the added protein (100 ng) contained truncated Pot1p as well as full length protein. Truncated Pot1p-DNA complex is indicated by a closed arrow.

On page 44 and continuing on to page 45, delete the entire paragraph beginning with "*Example 4*:" and replace this paragraph with the following in accordance with 37 C.F.R. §1.121. A marked up version showing changes is attached:

Example 4: Cloning of the hPOT1 gene.

Oligos PBoli164T (SEQ ID NO: 42) (TTCAGATGTTATCTGTCAATCAGAACCTG) and PBoli194B (SEQ ID NO: 43) (GAACACTGTTTACATCCATAGTGATGTATTGTTCC) were used to amplify a 614 bp fragment of *hPOT1* from multiple tissue cDNA panels (Clontech) with Advantage 2 Polymerase mix in the buffer supplied by Clontech. Cycling parameters of touch-down PCR were 94°C for 5 s, 68°C for 120 s (32 cycles). The gene encoding glyceraldehyde phosphate dehydrogenase (*GAPDH*) was used as a positive control for the integrity of the cDNA sample and was amplified for 26 cycles with primers (SEQ ID NO: 44) TGAAGG-TCGGAGTCAACGGATTTGGT and (SEQ ID NO: 45) CATGTGGGCCATGAGGTC-CACCAC.

REMARKS

The amendments add SEQ ID NOS to comply with 37 C.F.R. §§ 1.821-1.825, and they do not add new matter. Applicants respectfully request entry of the amendments and examination on the merits.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date August 7, 2001

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